Protocol by Virginia Rich, vrich@mit.edu, graduate student in the DeLong Lab
Last modified 4/2/08

DNA Extraction Protocol from 25mm 0.2um filters
Low-throughput, using DNeasy columns

by V. Rich 2/10/07, used for extracting DNA for 454 sequencing

This protocol is adapted from the 96-well protocol I worked out for environmental samples, which is built on the lab Steripak filter extraction protocol and the Suzuki et al 2001 extraction protocol.

Lysis Buffer: (make fresh because of sucrose)
adapted from Steripak protocol, with addition of RNase

Final Concentration	For 20ml
40mM EDTA	1.6ml of 0.5M EDTA
50mM Tris (pH 8.3)	1.0ml of 1M Tris (pH 8.3)
0.73M Sucrose	5.13g of Sucrose

filter sterilize through 0.2um filter and split into 2 10ml aliquots:

1. With Lysozyme & RNase: right before use, add to one 10ml aliquot:

Final Concentration	For 10ml
1.15mg/ml Lysozyme	11.54ug lysozyme
200ug/ml RNase 100mg/ml	20ul RNase 100mg/ml

shake to dissolve thoroughly, then filter-sterilize again

2. With ProK:
weigh out minimum amount ProK, then add the appropriate amount of lysis buffer (from the second 10ml aliquot)

Final Concentration	For e.g. 10ml	For e.g. 600ul
10mg/ml	100mg ProK	6mg ProK

filter sterilize

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Protocol

Increasing the lysis volume to try to maximize extraction efficiency, and because I'm using the DNeasy columns not the 96-well plate, so can load larger volumes…

Step 1: Cell Lysis & RNA removal
- thaw filters on ice
- transfer each filter to screw-top, O-ringed eppendorf tube
- add 500ul lysis buffer to each tube
- incubate 37ºC for 30", rotating end-over-end at angle, for optimal mixing with minimal frothing

Step 2: Protein Degradation

adapted from lab Steripak protocol
- add 37.5ul of Proteinase K solution (10mg/ml made up in lysis buffer) to a final conc. of 0.65 mg/ml
- add 59.7ul 10% SDS to a final conc of 1%
- incubate at 55ºC for 2 hours, rotating end-over-end at angle

Step 3: DNA Purification Through Qiagen DNeasy Tissue Kit DNA-binding column
- add 600ul Buffer AL (=Buffer AL/E without the ethanol added)

mix thoroughly by vortexing

incubate 70ºC for 10"
- add 600ul 96-100% EtOH

mix by vortexing vigorously

check pH of lysate, must be pH <7 to get max binding efficiency
- pipet max possible onto spin columns

spin >6000 x g for 10"; discard flow-through
- pipet additional lysate aliquots onto spin columns

spin >6000 x g for 1"; discard flow-through and collection tube, transfer column to new collection tube
- Add 500ul Buffer AW1

spin >6000 x g 1"; discard flow-through
- Add 500ul Buffer AW2

spin >6000 x g for 3"; discard flow-through and collection tube, transfer column to new collection tube
- then, to dry columns, spin 20,000x g for 3"
- add 200ul pre-heated 70ºC Buffer AE or water

incubate 1" @ RT

spin >6000 x g 2" to elute
- repeat with a second 200ul, this will increase yield up to 25%

Step 4: Final DNA Clean-up by Size-Exclusion Columns
- transfer the eluted DNA to the Microcon 30
- rinse DNA with 500ul Ambion water
- add 20ul dilute TE, pipette up and down 20 times and transfer to a clean 96-well plate for storage

(optional: repeat with another 20ul to ensure all retrieved)